

## CLAIMS

1. A method for classifying pixels of a microarray image with observed intensities within a region of interest, the method comprising:

5 initially classifying pixels in the region of interest as either feature pixels or background pixels based on the intensities of the pixels; and

iteratively computing, for pixels within the region of interest, probabilities that the pixels are feature pixels and probabilities that the pixels are background pixels, based on pixel locations and intensities, and accordingly classifying the pixels as either feature pixels  
10 or background pixels.

2. The method of claim 1 wherein a feature-pixel and background-pixel classification is stored in a feature mask.

15 3. The method of claim 2 wherein the feature mask includes binary values corresponding to pixels in the region of interest, a first binary value indicating that a corresponding pixel is a feature pixel and a second binary value indicating that a corresponding pixel is a background pixel.

20 4. The method of claim 1 wherein classifying pixels in the region of interest as either feature pixels or background pixels based on the observed intensities of the pixels further includes:

determining a high pixel intensity and a low pixel intensity for the region of interest;  
determining an intermediate point between the high pixel intensity and a low pixel  
25 intensity;

classifying pixels with observed pixel intensities greater than or equal to the intermediate point as feature pixels and classifying pixels with observed pixel intensities less than the intermediate point as background pixels; and

iteratively reclassifying pixels based on an intermediate intensity between the mean  
30 intensity of feature pixels and the mean intensity of background pixels.

5. The method of claim 1 further including identifying hole pixels that are feature pixels surrounded by background pixels and background pixels surrounded by feature pixels and reclassifying hole pixels in order to increase the continuity of feature-pixel and background-pixel classification with respect to location within the region of interest.

5

6. The method of claim 1 wherein iteratively computing, for pixels within the region of interest, probabilities that the pixels are feature pixels and probabilities that the pixels are background pixels, based on pixel locations and intensities, and accordingly classifying the pixels as either feature pixels or background pixels further includes:

10

iteratively

computing intensity-based outlier feature-pixel statistics and outlier background-pixel statistics;

from the most recently computed intensity-based feature-pixel statistics and background-pixel statistics, determining, for each pixel, a Bayesian posterior probability  $P(F/i,x)$  that the pixel is a feature pixel and a Bayesian posterior probability  $P(B/i,x)$  that the pixel is a background pixel and classifying the pixel as a feature pixel when  $P(F/i,x) \geq P(B/i,x)$ ;

15

20

until either a maximum number of iterations are performed or until fewer than a threshold number of pixels are reclassified from feature-pixel to background-pixel and from background-pixel to feature-pixel status in the most recently executed iteration.

7. The method of claim 6

wherein the Bayesian posterior probability  $P(F/i,x)$  is calculated as

$$P(F/i,x) = \frac{P(F,i,x)}{P(i,x)} = \frac{P(i/x,F)P(F,x)}{P(i,x)} = \frac{P(i/x,F)P(F/x)P(x)}{P(i,x)};$$

25

wherein the Bayesian posterior probability  $P(B/i,x)$  is calculated as

$$P(B/i,x) = \frac{P(B,i,x)}{P(i,x)} = \frac{P(i/x,B)P(B,x)}{P(i,x)} = \frac{P(i/x,B)P(B/x)P(x)}{P(i,x)};$$

and wherein a pixel is classified as a feature pixel when

$$\frac{P(F/i,x)}{P(B/i,x)} \geq 1.$$

8. The method of claim 7 wherein Bayesian posterior probabilities  $P(F/i,x)$  and  $P(B/i,x)$  are calculated for each channel of a two-channel microarray, and a joint probability distribution function for two channels is then computed, which is then used for classifying  
5 pixels as feature pixels or background pixels.

9. Computer instructions encoded in a computer-readable medium that implement the method of claim 1.

10 10. A data structure containing a feature-pixel and background-pixel classification carried out by the method of claim 1 stored in a computer-readable medium.

11. A feature extraction program that includes a feature-location-and-size determination step that includes the method for classifying pixels with observed intensities within a region  
15 of interest of claim 1.

12. Data produced by the feature extraction program of claim 11, stored in a printed medium or a computer readable medium, or encoded in electromagnetic signals, and transferred to a remote location.

20

13 Data produced by the feature extraction program of claim 11, stored in a printed medium or a computer readable medium, or encoded in electromagnetic signals, and received from a remote location.

25 14. A feature-extraction system comprising:  
a means for receiving and storing a scanned image of a microarray;  
a gridding means for determining putative feature positions and sizes within the scanned image of the microarray;  
feature-mask-generating logic that classifies pixels as feature-pixels and background-  
30 pixels based on pixel locations and intensities;

preview-mode display logic that displays feature positions and sizes obtained from the generated feature mask, solicits feedback from a user, and corrects the feature positions and sizes; and

5 a feature extraction module that extracts signal data from the scanned image of the microarray following user acceptance of initial feature locations and sizes displayed in preview mode.

15. The feature-extraction system of claim 14 wherein the feature-mask-generating logic classifies pixels as feature-pixels and background-pixels based on pixel locations and  
10 intensities by:

initially classifying pixels in a region of interest as either feature pixels or background pixels based on the intensities of the pixels; and

iteratively computing, for pixels within the region of interest, probabilities that the pixels are feature pixels and probabilities that the pixels are background pixels, based on  
15 pixel locations and intensities, and accordingly classifying the pixels as either feature pixels or background pixels.

16. The feature-extraction system of claim 15 wherein a feature-pixel and background-pixel classification is stored in a feature mask.

20

17. The feature-extraction system of claim 15 wherein classifying pixels in the region of interest as either feature pixels or background pixels based on the observed intensities of the pixels further includes:

determining a high pixel intensity and a low pixel intensity for the region of interest;

25 determining an intermediate point between the high pixel intensity and a low pixel intensity;

classifying pixels with observed pixel intensities greater than or equal to the intermediate point as feature pixels and classifying pixels with observed pixel intensities less than the intermediate point as background pixels; and

30 iteratively reclassifying pixels based on an intermediate intensity between the mean intensity of feature pixels and the mean intensity of background pixels.

18. The feature-extraction system of claim 15 wherein iteratively computing, for pixels within the region of interest, probabilities that the pixels are feature pixels and probabilities that the pixels are background pixels, based on pixel locations and intensities, and  
 5 accordingly classifying the pixels as either feature pixels or background pixels further includes:

iteratively

computing intensity-based outlier feature-pixel statistics and outlier background-pixel statistics;

10 from the most recently computed intensity-based feature-pixel statistics and background-pixel statistics, determining, for each pixel, a Bayesian posterior probability  $P(F/i,x)$  that the pixel is a feature pixel and a Bayesian posterior probability  $P(B/i,x)$  that the pixel is a background pixel and classifying the pixel as a feature pixel when  $P(F/i,x) \geq P(B/i,x)$ ;

15 until either a maximum number of iterations are performed or until fewer than a threshold number of pixels are reclassified from feature-pixel to background-pixel and from background-pixel to feature-pixel status in the most recently executed iteration.

19. The feature-extraction system of claim 18

20 wherein the Bayesian posterior probability  $P(F/i,x)$  is calculated as

$$P(F/i,x) = \frac{P(F,i,x)}{P(i,x)} = \frac{P(i/x,F)P(F,x)}{P(i,x)} = \frac{P(i/x,F)P(F/x)P(x)}{P(i,x)};$$

wherein the Bayesian posterior probability  $P(B/i,x)$  is calculated as

$$P(B/i,x) = \frac{P(B,i,x)}{P(i,x)} = \frac{P(i/x,B)P(B,x)}{P(i,x)} = \frac{P(i/x,B)P(B/x)P(x)}{P(i,x)};$$

and wherein a pixel is classified as a feature pixel when

25  $\frac{P(F/i,x)}{P(B/i,x)} \geq 1.$

20. The feature-extraction system of claim 19 wherein Bayesian posterior probabilities  $P(F/i,x)$  and  $P(B/i,x)$  are calculated for each channel of a two-channel microarray, and a joint

probability distribution function for two channels is then computed, which is then used for classifying pixels as feature pixels or background pixels.